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Effect of feeding flax or linseed meal on progesterone clearance rate in ovariectomized ewes☆

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Abstract

Ovariectomized ewes ($n = 22$; 68.76 ± 2.34 kg initial body weight; 2.9 ± 0.1 initial body condition score) were individually fed one of three diets: (1) control (phytoestrogen-free; $n = 7$), (2) flax containing diet ($n = 8$), or (3) linseed meal (LSM) containing diet ($n = 7$) to investigate the rate of progesterone (P4) clearance. On day 20 of feeding (day 0 = initiation of treatment), a P4 releasing device (CIDR) was placed in the vagina and jugular blood samples were obtained prior to CIDR insertion and 15, 30, 60, and 120 min following CIDR insertion. Further, blood samples were obtained daily between days 21 and 24. On day 25, blood samples were retrieved prior to CIDR removal and 2, 5, 10, 15, 30, 60, 120, and 360 min following CIDR removal. There was no difference in initial or final body weight or body condition score and there were no time by diet interactions on P4 clearance. The fractional rate of P4 uptake measured prior to CIDR insertion through day 4 following insertion tended to be greater ($P = 0.07$) in LSM fed ewes (508.75 ± 71.37%/min) compared to flax (295.39 ± 66.76%/min) and control fed (287.54 ± 71.37%/min) ewes. Diet tended ($P = 0.10$) to influence P4 clearance rate when measured from prior to CIDR removal through 120 min following CIDR removal with LSM fed ewes having a greater (1.26 ± 0.2) fractional rate constant than flax (0.929 ± 0.09) and control fed (0.922 ± 0.09) ewes. Flax fed ewes also had more ($P < 0.01$) omega-3 fatty acids and total fatty acids in plasma. Reports of increased pregnancy rates in dairy cows fed flax may relate to P4 metabolism.

Keywords: Flax; Linseed meal; Progesterone; Sheep

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1. Introduction

Over the last 20 years, pregnancy rates have decreased as dairy cattle have been continuously selected for an increased milk production [1]. Decreased fertility may result from genetics, reproductive management, nutritional management and/or changes in the physiology of the lactating dairy cow [2–4]. Early embryonic loss accounts for the majority of pregnancy losses that occur [5] and has been attributed to low levels of circulating progesterone (P4) in the dairy cow [6]. Decreased P4 could be the result of a decrease in the size or function of the corpus luteum or an increase in the metabolism of P4 by the liver. High producing dairy cows have elevated dry matter intake which increases liver blood flow, thereby increasing the metabolic clearance rate of many hormones, including P4 [7,8]. Lactating dairy cows consuming whole flax had increased first service conception rates compared to control fed cows [9]. The phytoestrogen, secoisolariciresinol diglycoside (SDG), is found within the flax hull and its solvent extraction by-product linseed meal (LSM). Women consuming defatted flax had a longer luteal phase during the menstrual cycle [10]. Flax also contains high levels of omega-3 fatty acids which can serve as precursors to series-3 prostaglandins [11]. In addition, high fat diets have been shown to increase circulating levels of P4 [12]. It is our hypothesis that the reported increase in pregnancy rates in dairy cow fed flax may be due to the impacts of flax, or LSM, on P4 metabolism. The objective was to determine the effect of feeding flax, which contains SDG and omega-3 fatty acids, or LSM containing only SDG, on P4 clearance rate in ovariectomized ewes.

2. Materials and methods

2.1. Animal procedures

The NDSU Animal Care and Use Committee approved all procedures involving animals for the following studies (IACUC #A0602). Western whiteface multiparous ewes ($n = 22$) at similar body weight (68.76 ± 2.34 kg) and body condition score (2.9 ± 0.1; scale of 1–5; 1 = thin, 5 = obese) [13] underwent bilateral ovariectomy and upon recovery were fed a phytoestrogen-free (PE-free) diet for a minimum of 30 days. Ewes were individually fed and housed in 0.91 m × 1.2 m pens in a temperature controlled (12 °C) and ventilated facility for the duration of the study. Lighting within the facility was automatically timed to mimic daylight patterns. Using a completely randomized design, ewes were assigned to one of three treatments (Table 1) including: a control diet (PE-free; $n = 7$), or diets containing flax ($n = 8$), or LSM ($n = 7$). Diets were formulated to be isocaloric and isonitrogenous (Table 2). Ewes were fed to meet maintenance requirements based on metabolic body weight ($BW^{0.75}$) with at least 12.4% crude protein (CP) and 0.84 Mcal/kg NEm [14] (Table 3). Body weight was measured fortnightly and body condition score was recorded at the onset (day 0) and conclusion of the trial (day 25). Orts

Table 1
Ingredient composition and feedstuffs utilized in treatment diets

Item	Treatment		
	Control	Flax	LSM
Flax (%)	–	12	–
Linseed meal (%)	–	–	8.125
Beet pulp (%)	79	78	82.125
DDGS (%)	20	10	8.5
Corn oil (%)	1	–	1.25

Table 2
Formulated nutritive values for treatment diets

Item	Control	Flax	LSM
NEm (Mcal/kg)	0.85	0.85	0.84
CP (%)	12.58	12.49	12.51
EE (%)	3.83	5.94	2.79
Ca (%)	0.86	0.72	0.85
P (%)	0.42	0.36	0.35

Table 3
Proximate analysis and fatty acid composition of treatment diets

Item	Control	Flax	LSM
DM (%)	91.40	87.75	89.92
Ash (% DM)	8.04	4.65	6.93
CP (% DM)	13.10	12.52	12.43
NDF (% DM)	43.21	35.42	44.56
ADF (% DM)	23.68	16.82	23.85
EE (% DM)	4.32	7.26	3.50
IVDMD (% DM)	87.02	86.01	85.14
Ca (% DM)	0.85	0.70	0.85
P (% DM)	0.33	0.34	0.32
Fatty acids	Total fatty acid (%)		
	Control	Flax	LSM
16:0	15.90	9.91	14.76
18:0	1.62	3.15	1.84
18:1$n-9$	23.05	18.61	21.81
18:2$n-6$	47.78	25.25	43.13
18:3$n-3$	1.75	37.09	7.46
Other	0.80	0.63	0.58
Total FA (mg/g of DM)	39.49	63.56	33.58

were collected twice weekly, weighed and composited, and ground through a 2-mm screen. Feed samples and orts were analyzed for dry matter (DM), ether extract (EE), ash, N, Ca, P (methods 930.15, 989.04, 942.05, 990.02, 968.08, and 965.17, respectively) [15], ADF, and NDF (Ankom, Fairport, NY). In vitro dry matter disappearance (IVDMD) was determined on diet samples by a modified procedure of Tilley and Terry [16], in which samples were centrifuged and the supernatant fluid was discarded before the addition of pepsin (Table 3).

On day 20 of feeding (day 0 = onset of treatment), a progesterone (P4) releasing device (EAZI-BREED CIDR®, InterAg, Hamilton NZ) containing no less than 0.3 g of P4 was inserted into the vagina. Blood samples were collected via jugular venipuncture prior to the insertion of the CIDR and 15, 30, 60, and 120 min after CIDR insertion. A single jugular blood sample was also taken daily prior to feeding from each ewe on days 21–24. One additional sample was collected on day 21 into a Vacutainer tube containing heparin for fatty acid analysis. On day 25 catheters were placed in the jugular vein and one blood sample was obtained from all ewes prior to CIDR removal. Vaginally placed CIDR devices were then removed from the ewes and blood samples were collected at 2, 5, 10, 15, 30, 60, 120, and 360 min following the removal of the CIDR. Samples were centrifuged in a swinging bucket centrifuge at $3000 \times g$ for 30 min at 4 °C. Serum and plasma were stored at −20 °C until assayed for P4.

2.2. Progesterone analysis

Progesterone was analyzed using hormonal chemiluminescence technology (IMMULITE, Siemens, Los Angeles, CA). Serum samples from each time point were assayed in duplicate. Within each assay, low, medium, and high P4 pools were run in duplicate. The intra- and inter-assay CVs were 7.1 and 12.5%, respectively.

2.3. Fatty acid analysis

Feed was prepared for fatty acid analysis via direct transesterification [17] with methanolic-HCl [18] and freeze-dried plasma fatty acids were prepped for fatty acid analysis as outlined by Lake et al. [19]. Separation of fatty acid methyl esters was achieved by GLC (Model CP-3800, Varian Inc., Palo Alto, CA) with a 100-m capillary column (SP-2560, Supelco, Bellefonte, PA) and H_2 as a carrier gas at 1.0 mL/min for feedstuffs and 1.5 mL/min for plasma. Oven temperature was maintained at 120 °C for 2 min and then ramped to 210 °C at 6 °C/min. Oven temperature was then ramped to 250 °C at 5 °C/min. Injector temperature was 260 °C and flame ionization detector temperature was 300 °C. Identification of peaks was accomplished using purified standards (Sigma–Aldrich, St. Louis, MO; Nu-Chek Prep, Elysian, MN; Matreya, Pleasant Gap, PA). Fatty acids which were not identified by identification peak standards were grouped together and reported as "other".

2.4. Calculations and statistical analysis

Rate of P4 absorption utilized nine time points (0, 15, 30, 60, 120 min, and days 1–4) after CIDR insertion and was calculated by first performing the natural log (ln) transformation of the data points and calculating the regression line of time (min) versus ln data. The absolute value of the slope ($|\lambda|$) of the regression line was considered the rate of absorption for P4 [20]. Nine time points (0, 2, 5, 10, 15, 30, 60, 120, and 360 min) after CIDR removal were used to calculate the fractional rate constant of P4 clearance ($(A) = (A)_0 e^{-kt}$, where A is the reactant (P4), k is the first-order fractional rate constant, and t is the time) [21].

Data were analyzed utilizing the general linear model of SAS for a completely randomized design. Data are presented as least squares means. Means were separated via least significant difference and differences were considered significant with a P-value of ≤ 0.05. Comparisons were made to determine the effects of diet on fatty acid levels as well as clearance rate of P4 measured in ng/(mL time).

3. Results

There were no significant differences in initial body weight ($P = 0.21$; 68.8 ± 2.3 kg) and body condition score ($P = 0.89$; 2.9 ± 0.1) between three groups, and dietary treatment had no effect on the final body weight ($P = 0.91$; 69.6 ± 2.1 kg) and body condition score ($P = 0.60$; 2.9 ± 0.1).

3.1. Plasma fatty acids

Plasma concentration of 18:3$n-3$ was greater ($P \leq 0.05$) for flax fed ewes than either control or LSM (Table 4). No differences ($P > 0.05$) were observed for 18:0, 18:1$n-9$, 18:2$n-6$, CLA, 20:3$n-6$, 20:4$n-6$, or 22:6$n-6$; whereas total fatty acid concentration was greatest ($P \leq 0.05$) for flax compared to control or LSM. However, when expressed as a percentage of total fatty acids, all fatty acids except 18:0, CLA, 22:6$n-6$, and other fatty acids, were increased ($P \leq 0.05$) in flax fed ewes compared to LSM and control ewes. Furthermore,

Table 4
Plasma concentration (mg/g of freeze-dried plasma) of fatty acids and percent (g/100 g of total fatty acids) of total fatty acids in blood plasma collected on day 21 after initiation of control, flax, or LSM diets in ovariectomized ewes

Fatty acid	Control	Flax	LSM	SEM	*P*
Plasma concentration (mg/g of freeze-dried plasma) of fatty acids					
18:0	2.25	2.31	2.09	0.19	0.68
18:1*n* − 9	2.51	2.49	2.64	0.16	0.78
18:2*n* − 6	4.31	4.04	3.76	0.26	0.37
18:3*n* − 3	0.14[a]	1.05[b]	0.11[a]	0.06	0.01
CLA[a]	1.08	1.74	1.13	0.21	0.07
20:3*n* − 6	0.06	0.04	0.05	0.005	0.07
20:4*n* − 6	0.71	0.55	0.75	0.07	0.10
20:5*n* − 3	0.03[a]	0.20[b]	0.05[a]	0.02	0.01
22:6*n* − 6	0.25	0.15	0.12	0.08	0.53
Other	4.90[a]	6.61[b]	5.21[a]	0.32	0.01
Total FA	16.43[a]	19.41[b]	16.09[a]	0.84	0.02
Percent (g/100 g of total fatty acids) of total fatty acids					
18:0	13.65	11.96	12.91	0.80	0.35
18:1*n* − 9	15.18[a]	12.97[b]	16.54[a]	0.73	0.01
18:2*n* − 6	26.33[a]	20.78[b]	23.28[a]	1.03	0.01
18:3*n* − 3	0.92[a]	5.36[b]	0.73[a]	0.32	0.01
CLA[c]	6.63	8.84	7.03	0.98	0.26
20:3*n* − 6	0.34[a]	0.21[b]	0.34[a]	0.02	0.01
20:4*n* − 6	4.32[a]	2.92[b]	4.67[a]	0.32	0.01
20:5*n* − 3	0.20[a]	1.01[b]	0.32[a]	0.11	0.01
22:6*n* − 3	1.46	0.77	0.76	0.45	0.50
Other	29.92	34.06	32.46	1.14	0.06

[a,b]Means ± pooled S.E.M. within row differ; $P < 0.05$.
[a] CLA = 18:2*cis*-9, *trans*-11.

LSM did not differ ($P > 0.05$) from control for any of the fatty acids measured.

3.2. Progesterone absorption

When P4 absorption was calculated (%/min), there was a tendency ($P = 0.07$) for LSM fed ewes to have a greater uptake of P4 compared to control and flax fed ewes. The unprotected LSD revealed that the rate of absorption for LSM fed ewes was greater ($P = 0.04$; 508.75 ± 71.37 %/min) compared to flax (295.39 ± 66.76 %/min) and control fed (287.54 ± 71.37 %/min) ewes (Fig. 1).

3.3. Progesterone clearance rate

The fractional rate constant for P4 clearance from prior to CIDR removal to 120 min following CIDR removal tended to be affected by treatment ($P = 0.17$). Ewes fed LSM tended ($P = 0.10$) to experience a greater (1.26 ± 0.20) fractional rate constant than those fed flax (0.93 ± 0.09) or control diets (0.92 ± 0.09). Overall, treatment had no effect ($P = 0.41$) on P4 intercept (Fig. 2).

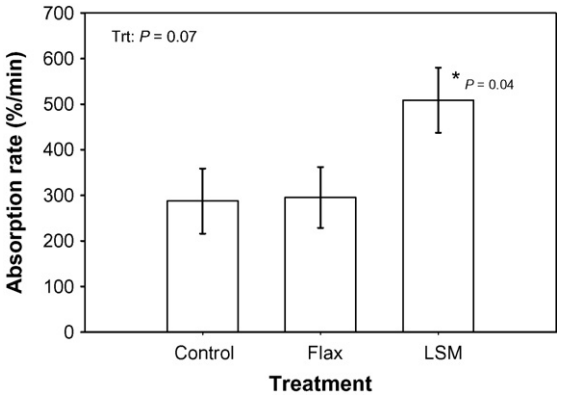

Fig. 1. Average rate of progesterone absorption (%/min) from CIDR insertion through day 4 after CIDR insertion. *F*-test tended to be different ($P = 0.07$). *, Unprotected LSD indicates that LSM > control = flax ($P = 0.04$).

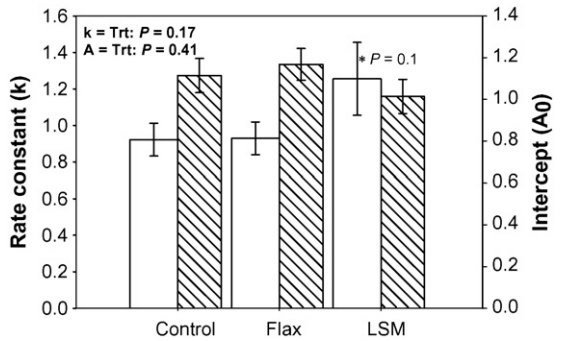

Fig. 2. Average rate constant (open bars) and intercept (cross-hatched bars) for P4 clearance in control, flax, and LSM ovariectomized ewes after CIDR removal. *F*-test tended to be different ($P = 0.17$). *, Unprotected LSD indicates that LSM tends to have a higher rate constant compared to control and flax fed ewes (*P*-value = 0.10).

4. Discussion

The mechanism by which inclusion of flax in the diet improves reproductive success in dairy cows may be due to a change in the rate of P4 clearance from circulation which has been observed with high fat diets [22]. In this study, the ovariectomized ewe was used as a model to determine how flax and/or LSM inclusion in the diet may impact P4 clearance. As there were no differences in weight gain or body condition, differences observed in progesterone uptake and clearance must be due to specific components in the diet. As endogenous estrogen can cause changes in the reproductive tract [23], it may be possible that the phytoestrogen, SDG, in LSM fed ewes increased blood flow and tissue permeability at the

site of the CIDR resulting in a greater uptake of P4 into the circulation. In high producing dairy cows inserted with CIDR devices, P4 metabolites in feces were greater in animals fed ad libitum compared to those restricted [24] indicating that daily P4 excretion may be related more to daily feed intake compared to the amount of P4 administered.

Perhaps increased concentrations of P4 alone may not impact fertility, as several studies supplementing P4 to sheep [25] and cattle [26] show no improvements on embryonic survival. Instead, it may be the alterations in an individual animal ability to metabolize progesterone. Phipps et al. [10] found that women consuming flax had a longer luteal phase during their menstrual cycle and higher P4 to estradiol ratios 1 week after ovulation, which was attributed to possible alterations in steroid metabolism. Ovariectomized ewes fed LSM had decreased liver weights compared to ewes of similar weight fed a phytoestrogen-free diet [27], indicating that LSM impacts the liver and/or liver function. Perhaps, LSM influences activity of the liver CYP enzymes [28], and the two major forms associated with P4 metabolism, CYP3A4 and CYP2C19, could be more active and would thus cause increased clearance of P4 from the circulation. In this study, feeding LSM to ovariectomized ewes tended to increase the clearance rate of P4. Since LSM contains mostly SDG, and relatively low amounts of omega-3 fatty acids, SDG could impact both the uptake and clearance of P4 in the ruminant.

The tendency for flax fed ewes to have lower clearance rates of P4 than LSM fed ewes may in part be explained by competition for enzymatic processes in the liver. The CYP enzymes discussed above may, in the case of flax fed animals, be down-regulated due to the association of peroxisome proliferator-activated receptors (PPARs), which can cause production of prostaglandins [29]. Since PPARs could be impacted by the availability of $n-3$ fatty acids such as 20:5$n-3$ [30] and flax fed ewes had greater plasma concentrations of 20:5$n-3$ than control or LSM, it may be possible that the tendency for flax fed ewes to have lower P4 clearance could be caused by increased activity of the PPAR enzyme. Fatty acids can serve as precursors to prostaglandins and dietary fat can increase circulating levels of cholesterol [31] and cholesterol-derived hormones [22,31]. The higher levels of plasma fatty acids in flax fed ewes may cause a shift from P4 metabolism, toward fatty acid redistribution and help contribute toward maintenance of pregnancy, particularly in cattle and sheep. The concentration of 18:2$n-6$ found in the plasma of control and LSM fed ewes was significantly greater than in ewes fed flax, which may lead to an increase in prostaglandin synthesis since 18:2$n-6$ is a precursor to arachidonic acid. Arachidonic acid is the major precursor to prostaglandin synthesis. Therefore, synthesis of PGE_2 is quite possibly influenced by fatty acid availability [32]. Greater availability of fatty acids may allow for greater production of PGE_2 and may impact uterine and ovarian function, which could affect the outcome of conception and pregnancy, particularly in the high producing dairy cow.

Feeding flax and/or LSM to livestock could be potentially beneficial for an improved fertility. In the current study, ewes fed LSM had greater absorption of P4 into the circulation from the CIDR. In addition, ewes fed flax tended to have lower clearance rates of P4 compared to LSM fed ewes, which could help decrease early pregnancy losses, particularly in dairy cattle, and thus reduce the costs associated with lost pregnancies.

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